REMARKS

Claim Amendments

Support for the claim amendments is found in the original application as follows.

Support for the amendments to the claims (e.g., Claim 1) referencing the coding region is found, for example, on page 35, line 34 to page 36, line 5; Example 5. The remaining amendments are clerical in nature and meant to clarify or simplify the claim language.

Support for new Claim 71 is found in Claim 1 as originally, previously presented and currently amended, and in the specification as previously discussed for this claim. Support for new Claim 72 is found in original Claim 10 and previously presented Claim 56. Support for new Claim 73 is found in Claim 1 as previously presented and presently amended and in previously presented Claim 50. Support for new Claims 74 and 75 is found on page 15, lines 13-18. Support for new Claim 76 is found on page 28, lines 19-21.

Nonstatutory Double Patenting

The Examiner has rejected Claims 40-47, 51 and 53-70 under the judicially created doctrine of obviousness-type double patenting, claiming that these claims are unpatentable over Claims 1-9, 13-15, 26-33, 37-39 and 50 of U.S. Patent No. 6,372,457.

To expedite prosecution, Applicants submit herewith a terminal disclaimer, which disclaims the terminal portion of the term of a patent issuing from the present application that would extend beyond the term of U.S. Patent No. 6,372,457. In view of this submission, Applicants respectfully request that the Examiner withdraw the obviousness-type double patenting rejection.

Rejection of Claims 40-70 Under 35 U.S.C. § 101

The Examiner has rejected Claims 40-70 under 35 U.S.C. § 101, contending that these claims are directed to non-statutory subject matter. The Examiner asserts that the claims, as written, do not sufficiently distinguish over nucleic acids, proteins, cells or antibodies as they exist naturally, because the claims allegedly do not point out any differences between the claimed products and naturally occurring products. The Examiner suggests that the claims be amended to recite a "transformed" or a "recombinant" microorganism.

Applicants traverse this rejection. Initially, it is noted that Claims 40-66 and 70 are **method** claims that recite the culture of a microorganism in a fermentation medium (step (a)) and the recovery and purification (step (b)) of a product from the fermentation medium. These claims are not product claims, and clearly represent statutory subject matter, *even if* they included a wild-type microorganism that could be obtained from nature. "New and useful processes" are statutory subject matter, according to 35 U.S.C. § 101. For these claims, there is no need to recite a "transformed" or "recombinant" microorganism and indeed, a genetically modified microorganism used in the claimed method need not be produced by transformation with a recombinant nucleic acid molecule.

With regard to Claims 67-69, Claim 67 clearly recites "said microorganism being transformed". However, to expedite prosecution, Claims 67-69 have been amended to recite a recombinant microorganism.

In view of the foregoing remarks, Applicants respectfully request that the Examiner withdraw the rejection of Claims 40-70 under 35 U.S.C. § 101.

Objection to the Specification and Rejection of Claims 40-70 Under 35 U.S.C. § 112, First Paragraph

The Examiner has objected to the specification and rejected Claims 40-70 under 35 U.S.C. § 112, first paragraph, on the basis of enablement. Specifically, the Examiner contends that the specification does not provide guidance for making any microorganism comprising any genetic modification that increases glucosamine-6-phosphate synthase action or any modification to the nucleic acid sequence encoding glucosamine-6-phosphate synthase. The Examiner asserts that the amount of experimentation required to obtain the claimed microorganism is undue, since it would include selecting from a variety of genetic modifications such as replacing the wild-type promoter of the synthase, modifying the nucleic acid sequence encoding the synthase, and selecting proteins other than the synthase to modify. The Examiner also contends that routine experimentation in the art does not include making a vast number of mutants and screening and selecting such mutants that have increased glucosamine-6-phosphate synthase activity, and that such selection is unpredictable. The Examiner asserts that one must know the specific type of modification or the amino acid residues to be modified. Finally, the Examiner contends that the Declaration of Ming-de Deng of

February 2002 does not provide enablement because the use of glucosamine-6-phosphate synthase-encoding genes from one additional bacterium and two different yeast does not teach how to make any microorganism comprising any genetic modification as claimed.

Applicants traverse the Examiner's rejection under 35 U.S.C. § 112, first paragraph. Initially, Applicants note that Claim 40 (and new Claim 72) has been amended to clarify the claim language by specifying that the genetically modified microorganism is modified in one of two ways: (1) by transformation of the microorganism with a recombinant nucleic acid molecule encoding a glucosamine-6-phosphate synthase (also recited in new Claim 71); or (2) by modification of the *coding region* of a gene encoding glucosamine-6-phosphate synthase selected from a deletion, insertion, and substitution of at least one nucleotide in the coding region. Claim 70 further describes one embodiment of the second method by reciting the classical strain development process by which such modifications can be generated and identified. Therefore, the claims are limited to microorganisms that have been recombinantly modified to overexpress a glucosamine-6-phosphate synthase, or that have been genetically modified in the coding region of a glucosamine-6-phosphate synthase gene by a specific type of modification, or that are identified using the genetic manipulation and classical strain development method that is illustrated through working examples by the specification. Applicants submit that the specification teaches one of skill in the art how to make and use such microorganisms and is therefore fully enabling.

More particularly, the specification teaches that the "[d]evelopment of a microorganism with enhanced ability to produce *N*-glucosamine by genetic modification can be accomplished using both classical strain development and molecular genetic techniques" (page 33, lines 29-32), and further describes in a detailed description and by working example how to produce such microorganisms using both molecular genetic techniques (e.g., pages 34-41 and Example 2) and classical strain development (e.g., page 10, lines 13-26; pages 13-14; page 33, lines 29-32; Examples 1 and 5-6). Applicants submit that Claim 40 and Claim 67, as presently amended, do not recite *any* microorganism with *any* modification that results in increased glucosamine-6-phosphate synthase activity but rather, *specific* modifications that are fully enabled by the specification and the skill in the art at the time of the invention.

With regard to the Examiner's comments regarding the Declaration of Dr. Deng, Applicants submit that this Declaration provides evidence that is directly relevant to the Examiner's prior comments during the telephone interview of August 21, 2001, wherein the Examiner indicated that additional data in support of the use of glucosamine-6-phosphate synthases other than the *E. coli* glucosamine-6-phosphate synthase would be useful in demonstrating support for the claimed genus of glucosamine-6-phosphate synthases. The specification is clear that any glucosamine-6-phosphate synthase can be recombinantly expressed or modified in the microorganism of the present invention (e.g., see page 15, lines 10-18). The Declaration of Dr. Deng clearly provides strong evidence that any glucosamine-6-phosphate synthase gene can be used for glucosamine production in any host using the claimed method of the present invention. It is noted that part (a) of Claims 40, 70 and 72, and new Claim 71 require only that the glucosamine-6-phosphate synthase be expressed recombinantly in the microorganism.

Moreover, the prior evidence of record further supports Applicants' position that, at the time of the invention, one of skill in the art would be able to determine where to modify a glucosamine-6-phosphate synthase and avoid destruction of enzyme activity, if desired. As discussed in paragraph 4 of the Declaration of Dr. Deng, by the end of 1997 (and therefore at the time of the present invention), genes encoding glucosamine-6-phosphate synthase, also known as glucosamine:fructose-6-phosphate amidotransferase, had been identified in many different organisms, including Gram negative bacteria, Gram positive bacteria, yeast, nematode, mouse and human. The Declaration further teaches that, even with low sequence homology, there are conserved structural and biochemical features among the glucosamine-6-phosphate synthases that result in similar function of the enzymes, and the Declaration specifies many of these features in paragraph 4. Therefore, one of skill in the art would know where to modify the enzymes without destroying function of the enzyme and would readily be able to select modifications increase activity without undue experimentation without undue experimentation (see further discussion below).

Finally, the specification teaches those of skill in the art how to produce genetically modified microorganisms with increased glucosamine-6-phosphate activity without the knowledge of where in the sequence the mutations occur, thus demonstrating that it is not necessary to know where to modify the sequence in order to produce the recited microorganisms and use them in the claimed

method. With regard to the Examiner's contention that routine experimentation does not include making a vast number of mutants and screening and selecting those that have the desired phenotype of increased glucosamine-6-phosphate synthase activity, Applicants strongly disagree. To the contrary, the Examiner should be aware that this is precisely how biologists have been producing and selecting desired mutants for many years. The predictability of producing a microorganism meeting the claim requirements and using the guidance in the specification is quite high, and routine experimentation is all that is required to produce, screen and select *multiple* microorganisms having the recited genetic modification. The advent of recombinant technology simply can not be used by the Examiner to dismiss classical techniques that have been used by investigators for years.

As taught in the specification, the genetically modified microorganisms of the present invention can be produced using classical mutagenesis techniques and/or molecular biology techniques (see page 10, lines 13-26; page 33, lines 29-32; Examples). The Examiner appears to contend that the only method of producing and selecting a microorganism of the present invention is by prior knowledge of precisely which amino acid modifications will produce the desired phenotype, but the specification demonstrates that such knowledge is not required. Once a mutation is discovered, the sequence information can be determined and used, if desired, to produce the same mutation using molecular biology. However, this prior knowledge is simply not necessary for one of skill in the art to make and use the present invention. In support of this position, the Examiner's attention is respectfully directed to the Examples 5-6.

In Example 5, in the investigation of transformed microorganisms, 4368 transformants were initially tested for glucosamine production. The screening of 4368 transformants is routine in this art, and was done in a single experiment. Initially, *96* candidates were selected as being producers of excess glucosamine (*i.e.*, glucosamine that significantly and measurably accumulates) as compared to the parent strain. Of these, approximately *one third* (*i.e.*, 30) were further selected as being superior glucosamine producers (*i.e.* resulted in larger halos of the indicator strain). The present inventors then selected six of these 30 for further investigation, although it is submitted that the selection of six was an arbitrary number (*i.e.*, all 30 or even all 96 could have been further investigated, without undue experimentation). Therefore, it should be clear that it is not

unpredictable to produce and select mutants meeting the claim requirements of increased glucosamine production.

Furthermore, of the six selected strains, three, or 50% had the expected phenotype of reduced feedback inhibition as compared to the wild type strain. Moreover, all three of these strains produced and accumulated significantly higher levels of glucosamine when the mutations were placed in a production background (See Example 6). If one wished to sequence the glucosamine-6-phosphate synthase-encoding nucleic acid molecules in any of the selected strains, this would be a routine step and would primarily be educational, and not a necessary step, since 96 microorganisms meeting the limitations of microorganisms useful in Claim 40 were produced without this information. In summary, from these data, it can be inferred that in any given single screen, multiple mutants can be identified that meet the claim limitations of having increased glucosamine production, and about half of the mutants that come through the initial plate screen can be predicted to have increased glucosamine-6-phosphate synthase action due to reduced feedback inhibition that would result in increased glucosamine production and accumulation. Applicants submit that this is a significantly high number and quite predictable, in contrast to the Examiner's assertion. The data presented in Examples 5-6 demonstrate that it is not required that any particular amino acid be targeted for mutation in order to produce a microorganism meeting the claim limitations. If one wishes to determine the identity of the mutation after the microorganism is identified by sequencing, this is a routine task. As discussed above, such sequence information could then be used to "tailor-make" a given mutant, if desired, but it is not necessary to use targeted mutational strategies to make and use the present invention. Applicants specification describes in detail how to make and identify the claimed microorganisms, and it is submitted that the requirements for enablement have been satisfied.

Furthermore, Applicants reference the case of In re Wands (858 F.2d 731, 8 USPQ 2nd 1400 (Fed. Cir. 1988)), and submit that this case supports Applicants' position. In In re Wands, the inventors had produced multiple antibody-secreting hybridomas. A few were selected for further analysis (out of a total of 143 hybridomas that were initially selected) and the remainder of the hybridomas identified in the initial screen were frozen. Of the nine antibodies selected for further analysis, four fell within the claims. In rejecting the claims on the basis of enablement, the board

argued that only 4 out of 143 hybridomas, or 2.8 percent, were *proved* to fall within the claims. However, the court upheld the Applicants' position, that the proper viewing of the data was that only 9 of the 143 were *selected* to be screened, and of these, a respectable 44% fell within the claims. "The PTO's position leads to the absurd conclusion that the more hybridomas an applicant makes and saves without testing the less predictable the applicants' results become." In summary, the court found that the Wands' disclosure provided "considerable direction and guidance on how to practice their invention and presents working examples. There was a high level of skill in the art at the time when the application was filed and all of the methods needed to practice the invention were well known." Furthermore, it was noted that "in the monoclonal antibody art it appears that an "experiment" is not simply the screening of a single hybridoma, but rather the entire attempt to make a monoclonal antibody against a particular antigen....Wands carried out this entire process three times, and was successful each time in making at least one antibody that satisfied all of the claim limitations."

Applicants submit that the facts in the present application mirror the facts in the Wands case. Classical strain development and genetic manipulation of microorganisms as well as molecular biological techniques to make the claimed mutations were well known in the prior art at the time of the invention. As discussed above, the instant specification provides considerable direction and guidance on how to practice the present invention, combined with several working examples. It is well known in the art of microorganism mutant production that a single experiment is not simply the screening of a single microorganism, but is rather the entire process of producing and screening *many* mutants (i.e., thousands) for the desired phenotype. The present inventors carried out the entire process in the present specification, and were successful in making at least one microorganism (actually several more than one) that satisfied all of the claim limitations. The present inventors have shown in Examples 5-6 that the required mutants can be obtained through routine experimentation, have provided the methods to do so in detail so that no additional guidance would be necessary to obtain the required mutants. It is not necessary to have prior knowledge of the necessary amino acid changes to obtain mutants with the desired properties.

In view of the foregoing remarks, Applicants respectfully request that the Examiner withdraw the rejection of Claims 40-70 under 35 U.S.C. § 112, first paragraph.

Objection to the Specification and Rejection of Claims 40-70 Under 35 U.S.C. § 112, First Paragraph:

The Examiner has objected to the specification and rejected Claims 40-70 under 35 U.S.C. § 112, first paragraph, on the basis of written description. Specifically, the Examiner contends that Claims 40-70 are genus claims that are directed toward any microorganism comprising any genetic modification that increases glucosamine-6-phosphate synthase activity. The Examiner contends that the genus is highly variable because a significant number of structural differences between the genus members is permitted. The Examiner asserts that the specification fails to describe additional representative species of the claimed genus other than transforming *E. coli* synthases into a host cell. With regard to the Declaration of Ming-de Deng, the Examiner contends that since the Declaration states that the nucleotide sequences of the genes from *B. subtilis*, *C. albicans*, and *S. cerevisiae* have low homology to the *E. coli* gene, then the *E. coli* synthase gene is not representative of the genus because there are allegedly no common structural features between the members of the genus.

Applicants traverse the Examiner's rejection of the claims under 35 U.S.C. § 112, first paragraph. Applicants submit that, contrary to the Examiner's contention, the specification provides sufficient guidance, combined with the knowledge in the art at the time of the invention, to demonstrate the inventors' possession of the invention as claimed. The specification clearly describes the use of any glucosamine-6-phosphate synthase in the claimed invention (e.g., see page 15, lines 10-18). First, Applicants submit that the structural characteristics (*i.e.*, the sequences) of glucosamine-6-phosphate synthases from a variety of organisms, including several microorganisms, were known in the art at the time of the present invention. Moreover, it was known in the art that there is a significant level of structural homology between the glucosamine-6-phosphate synthases from different organisms that relates to function. Structural homology does not necessarily equate to *overall* sequence homology; this is well known in the art of enzyme structure-function relationships (see discussion of cytochrome C below). In support of this position, Applicants enclose herewith publications that show glucosamine-6-phosphate synthase sequence information for various organisms and align the sequences to show the structural homology. For example, Fernandez-Herrero et al. (1995, *Mol. Microbiol.* **17(1)**:1-12), copy enclosed, compares the sequences of glucosamine-6-phosphate synthases of *E. coli, Rhizobium leguminosarum, Rhizobium meliloti,*

Thermus thermophilus, yeast, human and mouse. Similar sequences and alignments are shown in Leriche et al. 1997 (Eur. J. Biochem 245: 418-422), Smith et al. 1996 (*J. Bacteriol.* 2320-2327) and in McKnight et al., 1992 (*J. Biol. Chem.* 267:25206-25212). As shown in Figure 3 of Fernandez-Herrero et al., all of the enzymes contain key amino acid residues that are implicated as being mechanistically important for catalytic activity. This indicates a high degree of structural similarity even in the presence of low overall homology in this enzyme isolated from diverse sources. This structural information, coupled with the fact that the enzymes all catalyze the same activity indicates to one of skill in the art that they would all be useful for the production and accumulation of glucosamine and furthermore, because of the diversity of species that have been described, one could reasonably assume that a glucosamine-6-phosphate synthase from any source would be useful in the claimed method.

With regard to the Examiner's comments on the Declaration of Dr. Deng, Applicants submit that the Examiner is incorrect in concluding that because the Declaration states that the overall homology among the synthases is low, that the *E. coli* gene is not representative of the claimed genus. First, the claims are not limited to *E. coli* synthases or to synthases having a particular degree of homology to the *E. coli* synthases. The claims require the overexpression and/or modification of a glucosamine-6-phosphate synthase in a microorganism to produce and accumulate glucosamine-6-phosphate. The Examiner seems to ignore the discussion in the Declaration of Dr. Deng (see paragraph 4) that teaches that *despite* differences in sequence, all characterized glucosamine-6-phosphate synthases share many similar features that demonstrate a structure-to-function relationship.

Applicants submit that it is a widely accepted among biochemists and molecular biologists that a particular protein with a specific enzymatic activity can possess several different primary amino acid sequences and still maintain the enzymatic activity. It is often seen that only a few amino acids are "invariant" within a particular family of proteins with the same or similar enzymatic activities. These invariant amino acids often are part of a consensus sequence at the active site of the enzyme or at a particular structural domain(s) that is essential for enzymatic activity. At most other sites within the protein, it is typically the case that another amino acid can be substituted without having a significant effect on the activity of the enzyme. This is especially true when a

"conservative substitution" is made. That is, when an amino acid with similar biochemical properties (e.g., size, charge, hydrophilicity/hydrophobicity, side chains, etc.) is substituted, there is a good probability that there will be no significant negative effect on the activity of the resultant enzyme relative to the "wild-type" or "parent" enzyme.

Knowledge of the phenomena described above is widespread. Attached to this response are pages copied from a standard Biochemistry textbook (Voet and Voet, 2nd edition, 1995) that describe the wide spread existence of proteins with the exact or similar function, but with significant variability in primary amino acid sequence. The specific example highlighted in this textbook is cytochrome c, a protein that is essential to energy production in all eukaryotic organisms from yeast to mammals. Examination of this protein indicates that its amino acid sequence has been moderately conserved over evolutionary time. Examination of Table 6-4 on page 126 of the Voet and Voet textbook reveals that amino acid substitutions can be observed in nature in nearly one-half (**50%**) of the positions within the amino acid sequence while maintaining the enzymatic activity of the enzyme. Moreover, it is highly likely that additional amino acid substitutions could be made without an effect on the function of cytochrome c. This is because the cytochrome c molecules from only a small portion of the eukaryotic kingdom have been analyzed to date and because, as noted above, biochemists know that substitution of amino acids with similar properties are often well tolerated in regard to retention of enzymatic activities. Note that even in the limited number of cytochrome c sequences depicted in Table 6-4, there are individual sites at which six or seven different amino acids can substitute perfectly well.

Therefore, the Examiner is absolutely incorrect that there are no common structural features shared between members of the claimed genus, as evidenced by the prior submitted Declaration of Dr. Deng and the publications regarding glucosamine-6-phosphate synthase structure to function relationships available at the time of the invention. The fact that the various synthases have low overall homology and yet still function in the method of the invention is evidence that Applicants were in possession of the invention as claimed at the time of filing.

In view of the foregoing remarks, Applicants respectfully request that the Examiner withdraw the rejection of Claims 40-70 under 35 U.S.C. § 112, first paragraph.

Rejection of Claims 40-70 Under 35 U.S.C. § 112, Second Paragraph:

The Examiner has rejected Claims 40-70 under 35 U.S.C. § 112, second paragraph. Specifically, the Examiner contends that Claim 40 is indefinite for the phrase "encoding glucosamine-6-phosphate synthase which has glucosamine-6-phosphate activity". The Examiner asserts that the phrase is confusing since it is the enzyme encoded and not the nucleic acid itself that has activity.

In reply, although Applicants believe that the structure of the referenced phrase makes it clear that the phrase "which has glucosamine-6-phosphate activity" modifies the "glucosamine-6-phosphate synthase" directly preceding it and not the nucleic acid molecule itself (i.e., a nucleic acid molecule encoding a glucosamine-6-phosphate synthase which has glucosamine-6-phosphate activity), to expedite prosecution, Applicants have amended Claim 40 to clarify the phrase for the Examiner. Applicants note that Claims 67-69 do not depend from Claim 40 and therefore should not be included in this rejection. Applicants respectfully request that the Examiner withdraw the rejection of Claims 40-70 under 35 U.S.C. § 112, second paragraph.

Rejection of Claim 67 Under 35 U.S.C. § 102(b):

The Examiner has rejected Claim 67 under 35 U.S.C. § 102(b), contending that this claim is anticipated by Dutka-Malen et al. Specifically, the Examiner contends that Dutka-Malen et al. make a genetically engineered *E. coli* host cell that is transformed with a nucleic acid molecule encoding glucosamine-6-phosphate synthase. The Examiner considers that the linking of the nucleic acid sequence in the recombinant vector to a *lac* promoter constitutes a mutation to the glucosamine-6-phosphate gene.

Applicants traverse the Examiner's rejection of Claim 67 under 35 U.S.C. § 102(b). Initially, to clarify the claimed invention, Claim 67 has been amended to more clearly recite that the genetic modification is in the sequence encoding the synthase (i.e., the coding region of the nucleic acid molecule). Dutka-Malen et al. do not teach or suggest any modification of the coding sequence of the synthase, and therefore, Dutka-Malen et al. do not teach or suggest the invention as claimed in Claim 67. In view of the foregoing remarks, Applicants respectfully request that the Examiner withdraw the rejection of Claim 67 under 35 U.S.C. § 102(b).

Applicants have attempted to address all of the Examiner's concerns as set forth in the June 30 Office Action and submit that the claims are in a condition for allowance. If the Examiner has further concerns regarding Applicants' position, he is encouraged to contact the below-named agent at (303) 863-9700 to expedite prosecution.

Respectfully submitted,

SHERIDAN ROSS P.C.

By: Angela Sebor

Angela Dallas Sebor
Registration No. 42,460
1560 Broadway, Suite 1200
Denver, CO 80202-5141
(303) 863-9700

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